

Effect of cattle slurry on the growth of spinach plants in Cd-contaminated soil

Filipa R. Pinto¹, Miguel P. Mourato^{2,*}, Joana R. Sales², David Figueiro², Luísa Louro
Martins²

¹ MARE - Politécnico de Leiria, Marine and Environmental Sciences Centre, Instituto
Politécnico de Leiria, Edifício CETEMARES, Av. Porto de Pesca, 2520-630 Peniche -
Portugal

² LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-
017 Lisboa, Portugal

* Corresponding author:

Miguel P. Mourato, mmourato@isa.ulisboa.pt, tel. +351 213653587

Pinto F.R., Mourato M.P., Sales J.R., Figueiro D., Martins L.L. 2020. Effect of
Cattle Slurry on the Growth of Spinach Plants in Cd-contaminated
Soil. *Communications in Soil Science and Plant Analysis* 51(10): 1370-
1381. <https://doi.org/10.1080/00103624.2020.1781156>

<https://www.tandfonline.com/doi/abs/10.1080/00103624.2020.1781156?journalCode=lcss20>

Abstract

In this work the effect of the addition of different amounts of cattle slurry (CS) to a Cd contaminated soil, was studied regarding its effect in spinach plants. Two levels of Cd contamination (2 and 10 mg/kg) and three levels of CS addition were evaluated (2.5, 5 and 10 g CS/100 g soil). Spinach was shown to be a tolerant species, able to accumulate relatively high amounts of Cd (up to 367.7 mg/kg in the leaves), exceeding the limits established by European regulations for leaf vegetables. The addition of 2.5 and 5 g CS/100 g to soil containing 2 mg/kg Cd did not reduce the uptake of this metal but allowed the plants to grow as much as the control. The addition of 10 g CS/100 g lead to a reduced Cd uptake but also to a lower plant growth compared to the lower CS levels. The combined effects of Cd and CS changed ion homeostasis in the plant, but without causing severe toxicity or deficiency effects.

Keywords: Cadmium; mineral content; spinach; manure; abiotic stress

1 Introduction

Contamination of agricultural soils is a worldwide problem that can be caused by the deposition of metals and metalloids due to the misuse of pesticides and fertilizers, application of industrial effluents as water source for irrigation of crop plants, mining and smelting activities, and emissions from industries and transport vehicles (Nagajyoti, Lee, and Sreekanth 2010). Cadmium has a relatively high solubility and is readily taken up by crop plants, contaminating the food chain (Choppala et al. 2014; Clemens et al. 2013). These compounds can accumulate in different body organs leading to potential adverse effects on human health. A high percentage of trace elements in human bodies living in urban areas resulted from consuming contaminated foods rather than air pollution. In this context FAO/WHO recommended, for Cd, a Provisional Tolerable Weekly Intake of 7 µg/kg body weigh per week (EFSA 2009).

Leaf vegetables are an important part of the human diet and growing plants in contaminated soils is an important pathway for entry of toxic pollutants into human body. Mean values of Cd contents in soils worldwide vary between 0.2 and 1 mg/kg but can be much higher in contaminated soils (Clemens et al. 2013). Naturally, the ability of plants to grow in contaminated soils is highly dependent on environmental conditions and plant species (Mourato, Reis, and Martins 2012).

Spinach is a highly consumed leafy vegetable and also an important source of nutrients. The Food and Agriculture Organization (FAO) estimates that in 2017 more than 27000 Mt of spinach was produced all over the world, the vast majority in China and this value underestimate the real production as it does not include small and familiar gardens (FAO 2015). Spinach is especially sensitive to metal contamination as is known to be able to grow healthily and at the same time accumulating high amounts of different metals (Pinto et al. 2017). In a study comparing the growth of nine different vegetables in waste-amended soils,

77 Atkinson et al. (2012) reported that spinach accumulated more heavy metals than other
78 vegetables (except lettuce that accumulated more Cd than spinach). Sinha et al. (2007) also
79 found that spinach plants had good yields in contaminated soils with high accumulation of
80 metals in the edible parts .

81 Several techniques are used to remediate soils contaminated with PTE (Mulligan, Yong, and
82 Gibbs 2001). Organic matter application is one of the lower cost techniques that can be used
83 to control metal mobility in soils that can also have the added benefit of a positive effect on
84 the growth and yield of crops, promoting the restoration of soils (Mohamed et al. 2010;
85 Morsch and Martins 1999). However the influence of organic substances on the availability of
86 the PTE depends on the nature of these metals, soil types and the organic matter properties
87 (Mohamed et al. 2010; Gautam and Agrawal 2019).

88 The humic substances, such as humic acid (HA), fulvic acids (FA) and humin are important
89 fractions of soil organic matter, and its metal-cation binding capacity plays an important role
90 in their mobility (Hernandez-Soriano and Jimenez-Lopez 2012). However, some conflicting
91 results have emerged, since organic matter can reduce the availability of metals to plants, but
92 can otherwise increase it in certain circumstances (Bai et al. 2012).

93 Li et al. (2008) describes how the incorporation of pig manure in a contaminated soil
94 decreased the concentration of available Cu and Cd by 76.1 % and 25.7 %, respectively. In
95 another study (Liu et al. 2009), the application of chicken manure decreased the concentration
96 of soluble Cd by 71.8–95.7 %, but increased the values of inorganic precipitated Cd and
97 organic-bound Cd.

98 The objective of this work is to study the effect of the addition of different amounts of cattle
99 slurry (CS), 2.5, 5 and 10 g CS/100 g soil, obtained from a Portuguese dairy farm near
100 Lisbon, to soils contaminated with cadmium, in relation to its effects in the growth of spinach
101 plants and ion homeostasis.

2 Materials and Methods

2.1 Plant material, growth conditions and Cd treatment

Spinach plants were first germinated in substrate cylinders (Jiffy-7) for 35 days, with regular watering, a 12h photoperiod at a temperature between 22 and 25 °C. The plants were then transferred to pots containing 2 kg of soil (three plants per pot), with two concentrations of Cd (designated LC – Low Concentration with 2 mg Cd/kg and HC – High Concentration with 10 mg Cd/kg) and four levels of Cattle Slurry with 0, 2.5, 5 and 10 g CS/100 g (designated CS0, CS2.5, CS5 and CS10, respectively). Including the four controls (No Cd and 0, 2.5, 5 and 10 g CS/100 g) and considering 4 pots per treatment, there were a total of 48 pots, in a completely randomized design.

The Cd containing soils had been previously contaminated with the adequate Cd concentrations (2 and 10 mg/kg), using a CdCl₂ solution, six months prior to the experiment. The CS was added two months prior to the experiment.

The soil was fertilized in three batches (just before cultivation and 26 and 33 days after planting) with 36.10 mg K/kg of soil (added as KNO₃), 4.09 mg P/kg of soil (added as KH₂PO₄) and 33.28 mg N/kg of soil (added as KNO₃ and Ca(NO₃)₂). The pots were irrigated periodically (to 70 % water capacity) and were kept in a greenhouse under normal daylight for the duration of the experiment. Seventy days after germination the plants were collected for further analysis, as described below. Soil samples were also collected at the same time.

2.2 Determination of spinach weight, essential elements and Cd content

Spinach leaves, stems and roots were collected and immediately weighed to obtain the fresh weight (FW). Samples were washed with deionized water and dried at 70 °C until constant weight to obtain the dry weight (DW).

Subsamples with 0.5 g were digested with 10 mL concentrated nitric acid in a digestion block (DigiPREP MS) for 100 min. The solutions were then analyzed for Cd, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) by atomic absorption spectrophotometry (Unicam Solaar M).

2.3 Soil analysis

Soil samples from each pot (ca. 100 g) were dried at 50 °C for 5 days in order to perform soil analysis. The soil used in this experiment was a sandy soil with a pH(H₂O) of 5.67±0.09, pH(KCl) of 5.05±0.10, <0.01% organic matter, and <5 mg/kg K and P (as K₂O and P₂O₅, respectively). Nitrogen content was using a segmented flow auto analyzer (Skalar). The sample solution was obtained by stirring for 60 min, 6 g of dry soil with 30 mL of 2 M KCl, followed by a 10 min centrifugation at 3500 rpm. The supernatant was then transferred to the reading tubes. Phosphorus and K were extracted using a solution of ammonium lactate and acetic acid buffered to pH 3.75. To 2 g of dried soil was added 40 ml of extraction solution and stirred for 2h. The solution was filtered and K was quantified by flame emission photometry. Phosphorous was determined by adding to 10 mL of sample solution, 2 ml of molybdcic photo-rex reagent and 1 mL solution of stannous chloride in HCl 6 M. After allowing the colour to develop for 30 min, the absorbance was read in a molecular absorption spectrophotometer at 775 nm.

For the determination of exchangeable bases (Ca, Mg, K and Na), 2 g of dry soil were stirred with 30 mL of ammonium acetate 1 M (pH 7) over 15 minutes. After centrifugation for 10 min at 3500 rpm, each element was quantified by flame atomic absorption spectrophotometry (Unicam Solaar M). The micronutrients Cu, Fe, Zn and Mn were determined by stirring 4 g of dry soil with 40 mL of extracting solution (acetic acid 0.5 M, ammonium acetate 0.5 M and

EDTA 0.02 M) over 15 minutes. After centrifugation for 10 min at 3500 rpm, each element was quantified by flame atomic absorption spectrophotometry (Unicam Solaar M).

Soil pH was determined in a soil/water suspension (pH H₂O) and soil/solution of 1M KCl (pH KCl) in the proportion 1: 2.5 (w/v), after 1 hour of contact, using a Metrohm pH meter model 632. Total organic carbon (TOC) was determined in a Skalar TOC Analyzer, using 1.5 g of dry soil. The soil characteristics are presented in Table 1.

2.4 Cattle Slurry Analysis

The CS used was collected from a dairy farm near Lisbon, Portugal, where the animal food was based on feed and silage. The CS was preserved at 4 °C in plastic barrels before application. The CS Characteristics (dry matter, organic matter, pH, conductivity, total N, total P and content of K, Na, Mg, Ca, Fe, Cu, Zn and Mn) were determined following the procedures described by Fangueiro et al. (2009) and are presented in Table 2.

2.5 Statistical analysis

Statistical analysis was performed using the software SPSS 13.0 (SPSS Inc, 1989-2004). The results were subjected to a one-way ANOVA using the Tukey test to check for significant differences between means ($P < 0.05$). Error bars in figures and tables represents standard deviation. All the experimental determinations described above were performed in triplicate.

3 Results

Spinach shoot fresh mass obtained at the end of the experiment, 70 days after germination (DAG), is presented in Fig. 1. The highest amount of biomass was obtained for spinach plants growing in non-contaminated soil with CS5 (7.1 g per plant), while the lowest value (0.3 g per plant) was measured in the Cd-contaminated soils (10 mg/kg) with CS10. No significant

differences were detected between the control and LC soils, but plant growth was significantly affected in HC soils for all the tested CS concentrations.

In Table 3, Cd content in the different plant parts is presented. In the leaves the maximum Cd content was 367.7 mg/kg DW in HC soil with CS2.5, while at higher CS levels the amount of Cd was reduced. In the roots, the maximum measured Cd content was 520.5 mg/kg DW in HC soil with CS5, although this was not significantly different from the CS2.5 treatment.

In Fig. 2 the percentage of total Cd that is accumulated in the leaves, stems and roots for both Cd concentrations is presented. It can be seen that with higher CS content there is a larger percentage of Cd accumulating in the roots and a lower percentage in the leaves.

The effect of Cd contamination and CS application in the uptake of other essential elements is shown in Table 3 for roots and leaves, for Na, K, Ca, Mg, Fe, Cu, Mn and Zn. There is a large variation in the levels of the different elements but globally the changes are not enough to cause deficiency or toxicity symptoms.

4 Discussion

It is known that organic matter (OM) incorporation in soils can cause a positive effect on the growth and yield of crops (Mohamed et al. 2010; Sinha et al. 2007). Our results indicate an increase in biomass production for plants grown in both contaminated and non-contaminated soils with CS2.5 and CS5 (Fig. 1) compared to the control. In the contaminated soils, although there was a decrease in biomass with increasing Cd content, the presence of CS2.5 and CS5 was shown to have a positive effect in avoiding the Cd toxic effect. It is evident that both CS2.5 and CS5 can counteract part of the harmful effect of Cd, mainly at the lower concentration of 2 mg/kg. This is no longer valid for the highest Cd concentration in soil, since the optimum ratio of OM/Cd could be exceeded.

200 One possible cause for the positive effect of the added CS to the soil was the increase in pH
201 (Table 2). Spinach plants grow better at a pH around or above 7, and as the pH of the initial
202 soil was acidic the differences between the control and CS2.5 and CS5 experiments can be
203 partially explained by the pH increase. The added CS, at those levels, did also improve
204 nutrient provision for the plants, facilitating its growth (Beesley et al. 2014). With the CS10
205 treatment the biomass production reverts to the control levels and the beneficial effects in
206 plant growth observed for the lower CS levels are not visible anymore. This reduction in
207 biomass production with increased organic matter can be due to the nature of the organic
208 matter (Inaba and Takenaka 2005) and other different factors and has been reported also by
209 other authors (Narwal and Singh 1998; Bai et al. 2012). As can be seen in Table 2, the soil
210 characteristics changed with the increase of CS, and a significant increase in pH and
211 conductivity was observed which could have an impact in plant production. Also, the C/N
212 ratio is important as higher values of this parameter could cause the immobilization of
213 nitrogen (and also of phosphorous), affecting plant productivity. However, the C/N ratio of
214 the CS used in this study was much lower than in the organic matter used by other authors
215 (Bai et al. 2012). In this last study, in rice plants, the authors also observed a decrease in plant
216 biomass with increasing levels of straw applied to the soil. They also attributed the decrease
217 in biomass to the putative effect of toxic substances in the organic matter, and this might also
218 be a factor to explain the results obtained in this work.

219 The results presented in Table 3 confirm that the toxic effect observed in the CS10
220 experiment was not due only to Cd uptake by spinach plants. In fact, only at this
221 concentration of CS was observed a significant decrease in Cd uptake in all the analyzed plant
222 parts. The addition of CS2.5 and CS5, for both Cd concentrations studied (LC and HC), does
223 not cause a significant decrease in the uptake of Cd by spinach plants. However, these levels
224 of CS are sufficient to improve the biomass production of the plant indicating that the positive

225 factors that affect plant growth overcome the toxic effect induced by Cd. Actually, a
226 significant increase in the uptake of Cd by the roots was measured at both CS2.5 and CS5
227 treatments. This might be due to increased solubility of Cd in the presence of increased
228 amounts of dissolved carbon, as has been reported earlier (Antoniadis and Alloway 2002).
229 The increase in soil humic acids content by the addition of the CS can also increase Cd uptake
230 by plants as has been shown by other authors (Evangelou, Daghan, and Schaeffer 2004).
231 Another explanation could be the formation of soluble CdCl^+ complexes that lower the
232 affinity of Cd to the organic matter (Adriano et al. 2004). On the other hand, the opposite
233 effect was observed when the CS concentration reached 10%. As the metal concentration in
234 soil solution is dependent on pH and on the nature and amount of both organic and inorganic
235 ions (Bolan et al. 2014), the higher pH of the soil with CS10 can increase Cd chelation by
236 organic matter leading to a reduce Cd uptake by the plant (Adriano et al. 2004). The lower CS
237 concentrations apparently are not high enough to trigger this effect.

238 The level of CS also affected the distribution of Cd in the plant parts. In Fig. 2 the percentage
239 of total Cd that is accumulated in the leaves, stems and roots in relation to the total, is
240 presented. As can be seen, plants growing in soils containing a higher percentage of CS show
241 an increase in the levels of Cd in the roots concomitant with a decrease in the leaves, that is,
242 the translocation of Cd between roots and leaves decreased with higher CS%. This effect is
243 more pronounced in the HC treatment than in the LC. The explanation of this decreased
244 translocation, and thus of a higher Cd retention in the roots, is probably related to the
245 increased tolerance of spinach to Cd under higher levels of CS. We postulate that the addition
246 of CS to the soil leads to increased availability of sulphur compounds leading to higher
247 glutathione and phytochelatins (PC) synthesis in the roots (Khan et al. 2015). These
248 compounds are known to be very important in plant resistance to PTE toxicity, especially Cd,
249 as they can form complexes with this metal and transport them to the vacuoles (Seth et al.

2012). This can increase the tolerance of the plant, and can be another part of the explanation for the obtained results of better plant growth under higher Cd concentrations when grown in soils containing CS. Moreover, this can also explain the decreased translocation of Cd between roots and leaves, as an increase in PC synthesis will likely lead to increased retention of Cd-PC complexes in the root vacuoles. Pinto et al. (2004) reported an increased Cd accumulation in shoots in relation to roots of sorghum plants, with increased organic matter but these results were obtained in nutrient solution and the authors explained it due to a decreased Cd bioavailability.

As spinach is reported to be a relevant food source of mineral elements (Citak and Sonmez 2009) it is important to evaluate how the different growth conditions affect their concentrations. It has been reported that Cd can be taken up by the plants via the transporters of essential cations, like Ca, Zn, Fe, Mg and Cu, and thus affecting the element homeostasis in the plant (Gallego et al. 2012). However these effects are highly dependent on plant species and metal (Martins et al. 2013). In Table 3 the concentrations of essential elements in leaves and roots are presented.

As can be seen in this table, the levels of K decreased with Cd uptake. This effect is more pronounced in the roots, and in the leaves the plant manages to compensate K levels and only for the highest concentration of Cd is there a significant decrease in plants growing in CS-containing soil. However, in the control plants K levels in leaves are always lower in plants growing in Cd contaminated soils. As K has an osmotic function in plants, it may be substituted by Na in plants with considerable Na uptake potential, as has been reported for spinach (Mengel 2007). Our results are in agreement with this as we can observe an increase in Na levels in leaves of plants growing under Cd stress.

Although the levels of Ca decreased in leaves with increasing CS concentration, no reduction was detected due to Cd. On the contrary, an increase in Ca content was measured in spinach

leaves with CS2.5 and CS5, for the HC Cd treatment. As spinach plants showed reduced growth under this Cd concentration, the observed increase in Ca content could be due to disturbances in the plasma membrane leading to increased Ca influx, as has been proposed by Michalska and Asp (2001) that detected similar effects in lettuce growing under Cd and Pb stress. A similar effect occurs with Fe and Mn, with a generally increased element uptake under Cd stress, more pronounced in CS10 soils for iron and in CS0 or CS2.5 for Mn. This could also be due to a disturbance in the uptake mechanisms of this element that happens with plants growing under CS10 and HC Cd concentration. Although several Cd toxicity studies report decreases in the uptake of several ions (DalCorso et al. 2008), the opposite effects, similar to the ones described in this work, have also been reported (de la Rosa et al. 2004; López-Millán et al. 2009).

As for Mg, the presence of Cd led to a decrease in the root content of this essential element, leading to the conclusion that Cd is affecting Mg uptake mechanisms by the roots. This effect is also reflected in the Mg levels in the leaves that are generally lower under Cd stress. However, this reduction is not high enough to cause deficiency problems in the plant. In relation to Cu, an increase in its content in roots was observed, but not in leaves, confirming that the translocation to this organ was affected, mainly at the higher Cd concentration.

Zinc was the element most clearly affected by Cd. For all the CS contents, the uptake of Zn by the spinach roots was reduced under Cd toxicity, although the effect was less pronounced for the highest CS level. This translated in lower contents of Zn in leaves. Normal levels of Zn in spinach are within the values of 50 and 75 mg/kg DW, and for plants growing with the HC treatment the values are slightly below this lower limit but not low enough to induced serious deficiency effects. In another study with spinach plants in nutrient solution (Pinto et al. 2017), the same effect of apparent Cd competition with Zn was observed, confirming that for this plant, Cd uptakes affects the transport of Zn.

As can be seen in Table 3, although the roots showed the higher concentration of Cd, the above-ground parts, both stems and leaves, also accumulate considerable amounts of Cd, for both Cd treatments, LC and HC. The leaves of contaminated plants accumulated up to 367.7 ± 6.4 mg Cd/kg DW (corresponding 25.75 mg Cd/kg FW), exceeding the maximum value allowed to leaf vegetables (0.2 mg/kg FW) according to the European regulations (Commission Regulation (EC) 2006). Even at the lower Cd contamination evaluated in the present study spinach plants were able to absorb 12.91 mg Cd/kg FW, also exceeding the regulated value. Our results confirm that spinach has the potential to exceed these legal threshold levels, if the conditions (Cd concentration, growing medium and exposition period) are adequate. As such, the FAO recommended value of Provisional Tolerable Weekly Intake of 7 $\mu\text{g/kg}$ body weight (EFSA 2009) can be quickly overcome with the consumption of highly contaminated spinach, in a diet that includes moderate consumption of this vegetable. Moreover, the present study confirms that the addition of CS will lead to increased plant growth but also to increased Cd uptake, in a contaminated soil. Thus, the plants might even look healthier than those growing without CS but at the same time containing high amounts of Cd in its edible parts. This constitutes an increased food safety hazard as the vegetables will look healthy, reducing the probability of rejection by the consumer.

5 Conclusions

The growth of Spinach plants in Cd-contaminated soil containing different amounts of cattle slurry has demonstrated different responses according to the CS concentration. At the CS2.5 and CS5 treatments plant growth has improved but Cd uptake was not decreased, as the type of organic matter probably increased Cd mobility in the soil. A higher concentration of CS (10%) caused a significant decrease in Cd uptake but also a reduction of plant growth, presumably due to the high pH or high C:N ratio, or the presence of other substances in the

CS. This is evidence that there are different opposite factors at stake that are highly dependent on the level of CS applied to the soil.

Spinach plants with 70 days of development, at a similar development stage as used for human consumption, were able to tolerate Cd at a concentration of 25.75 mg/kg leaf FW while not showing visual evidences of stress, except for reduced biomass. These Cd levels are well above the maximum recommended values according to European regulations.

Spinach plants growing under Cd stress in different CS concentration showed affected essential metal ion uptake but not enough to cause either deficiency or toxicity problems. In reality an increase in the concentrations of some beneficial elements was detected in Cd-contaminated plants.

These results confirm that CS is not adequate to reduce the uptake of Cd by spinach plants as at the concentrations necessary for this decrease in uptake, other negative factors will prevail that will cause toxic effects and a decrease in the biomass production. Further studies on CS application to the soil should focus on possible corrective measures, like decreasing pH and changing the C:N ratio in order to improve CS ability to retain Cd in soil.

Acknowledgments

Filipa R. Pinto acknowledges funding from the Fundação para a Ciência e Tecnologia (FCT, Lisboa, Portugal) in the form of grant SFRH/BD/81080/2011. This work was partially funded by FCT Research Project PTDC/AGRAAM/102821/2008 and by the FCT-funded research unit LEAF - Linking Landscape, Environment, Agriculture and Food (UID/AGR/04129/2013).

6 Bibliography

- Adriano, D. C., W. W. Wenzel, J. Vangronsveld, and N. S. Bolan. 2004. "Role of assisted natural remediation in environmental cleanup." *Geoderma* 122:121-42.
- Antoniadis, V., and B. J. Alloway. 2002. "The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge-amended soils." *Environmental Pollution* 117 (3):515-21. doi: [http://dx.doi.org/10.1016/S0269-7491\(01\)00172-5](http://dx.doi.org/10.1016/S0269-7491(01)00172-5).
- Atkinson, N. R., S. D. Young, A. M. Tye, N. Breward, and E. H. Bailey. 2012. "Does returning sites of historic peri-urban waste disposal to vegetable production pose a risk to human health? - A case study near Manchester, UK." *Soil Use and Management* 28 (4):559-70. doi: 10.1111/j.1475-2743.2012.00438.x.
- Bai, Yanchao, Chuanhui Gu, Tianyun Tao, Guohua Chen, and Yuhua Shan. 2012. "Straw incorporation increases solubility and uptake of cadmium by rice plants." *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* 63 (3):193-9. doi: 10.1080/09064710.2012.743582.
- Beesley, Luke, Onyeka S. Inneh, Gareth J. Norton, Eduardo Moreno-Jimenez, Tania Pardo, Rafael Clemente, and Julian J. C. Dawson. 2014. "Assessing the influence of compost and biochar amendments on the mobility and toxicity of metals and arsenic in a naturally contaminated mine soil." *Environmental Pollution* 186 (0):195-202. doi: <http://dx.doi.org/10.1016/j.envpol.2013.11.026>.
- Bolan, Nanthi, Anitha Kunhikrishnan, Ramya Thangarajan, Jurate Kumpiene, Jinhee Park, Tomoyuki Makino, Mary Beth Kirkham, and Kirk Scheckel. 2014. "Remediation of heavy metal(loid)s contaminated soils – To mobilize or to immobilize?" *Journal of Hazardous Materials* 266 (0):141-66. doi: <http://dx.doi.org/10.1016/j.jhazmat.2013.12.018>.

374 Choppala, Girish, Saifullah, Nanthi Bolan, Sadia Bibi, Muhammad Iqbal, Zed Rengel, Anitha
375 Kunhikrishnan, Nanjappa Ashwath, and Yong Sik Ok. 2014. "Cellular Mechanisms in
376 Higher Plants Governing Tolerance to Cadmium Toxicity." *Critical Reviews in Plant*
377 *Sciences* 33 (5):374-91. doi: 10.1080/07352689.2014.903747.

378 Citak, Sedat, and Sahriye Sonmez. 2009. "Mineral Contents of Organically and
379 Conventionally Grown Spinach (*Spinacea oleracea* L.) during Two Successive
380 Seasons." *Journal of Agricultural and Food Chemistry* 57 (17):7892-8. doi:
381 10.1021/jf900660k.

382 Clemens, Stephan, Mark G. M. Aarts, Sebastien Thomine, and Nathalie Verbruggen. 2013.
383 "Plant science: the key to preventing slow cadmium poisoning." *Trends in Plant*
384 *Science* 18 (2):92-9. doi: <http://dx.doi.org/10.1016/j.tplants.2012.08.003>.

385 Commission Regulation (EC). 2006. "No 1881/2006 setting maximum levels for certain
386 contaminants in foodstuffs." *Official Journal of the European Union* L 173/6.

387 DalCorso, G., S. Farinati, S. Maistri, and A. Furini. 2008. "How plants cope with cadmium:
388 Staking all on metabolism and gene expression." *Journal of Integrative Plant Biology*
389 50 (10):1268-80. doi: 10.1111/j.1744-7909.2008.00737.x.

390 de la Rosa, Guadalupe, Jose R. Peralta-Videa, Milka Montes, Jason G. Parsons, Irene Cano-
391 Aguilera, and Jorge L. Gardea-Torresdey. 2004. "Cadmium uptake and translocation
392 in tumbleweed (*Salsola kali*), a potential Cd-hyperaccumulator desert plant species:
393 ICP/OES and XAS studies." *Chemosphere* 55 (9):1159-68. doi:
394 <http://dx.doi.org/10.1016/j.chemosphere.2004.01.028>.

395 EFSA. 2009. "Scientific Opinion of the Panel on Contaminants in the Food Chain on a
396 request from the European Commission on cadmium in food." *The EFSA Journal*
397 980:1-139.

398 Evangelou, M. W. H., H. Daghan, and A. Schaeffer. 2004. "The influence of humic acids on
 399 the phytoextraction of cadmium from soil." *Chemosphere* 57 (3):207-13. doi:
 400 10.1016/j.chemosphere.2004.06.017|ISSN 0045-6535.

401 Fangueiro, David, Henrique Ribeiro, Ernesto Vasconcelos, Joao Coutinho, and Fernanda
 402 Cabral. 2009. "Treatment by acidification followed by solid-liquid separation affects
 403 slurry and slurry fractions composition and their potential of N mineralization."
 404 *Bioresource Technology* 100 (20):4914-7. doi: 10.1016/j.biortech.2009.04.032.

405 FAO. "Food and Agriculture Organization of the United Nations, FAOSTAT database,
 406 available at <http://faostat3.fao.org/home/E>." <http://faostat.fao.org/>.

407 Gallego, Susana M., Liliana B. Pena, Roberto A. Barcia, Claudia E. Azpilicueta, Maria F.
 408 Lannone, Eliana P. Rosales, Myriam S. Zawoznik, Maria D. Groppa, and Maria P.
 409 Benavides. 2012. "Unravelling cadmium toxicity and tolerance in plants: Insight into
 410 regulatory mechanisms." *Environmental and Experimental Botany* 83:33-46. doi:
 411 10.1016/j.envexpbot.2012.04.006.

412 Gautam, Meenu, and Madhoolika Agrawal. 2019. "Effects of Red Mud Addition in Soil
 413 Fertilized with Cowdung Manure on Growth Performance and Metal Accumulations
 414 in Brassica juncea Cultivars Kranti and Pusa Bold." *Communications in Soil Science
 415 and Plant Analysis* 50 (10):1214-31. doi: 10.1080/00103624.2019.1614599.

416 Hernandez-Soriano, Maria C., and Jose C. Jimenez-Lopez. 2012. "Effects of soil water
 417 content and organic matter addition on the speciation and bioavailability of heavy
 418 metals." *Science of the Total Environment* 423:55-61. doi:
 419 <http://dx.doi.org/10.1016/j.scitotenv.2012.02.033>.

420 Inaba, Shoko, and Chisato Takenaka. 2005. "Effects of dissolved organic matter on toxicity
 421 and bioavailability of copper for lettuce sprouts." *Environment International* 31
 422 (4):603-8. doi: <http://dx.doi.org/10.1016/j.envint.2004.10.017>.

423 Khan, M. Iqbal R., Faroza Nazir, Mohd Asgher, Tasir S. Per, and Nafees A. Khan. 2015.
 424 "Selenium and sulfur influence ethylene formation and alleviate cadmium-induced
 425 oxidative stress by improving proline and glutathione production in wheat." *Journal*
 426 *of Plant Physiology* 173:9-18. doi: 10.1016/j.jplph.2014.09.011.

427 Li, Ping, Xingxiang Wang, Taolin Zhang, Dongmei Zhou, and Yuanqiu He. 2008. "Effects of
 428 several amendments on rice growth and uptake of copper and cadmium from a
 429 contaminated soil." *Journal of Environmental Sciences* 20 (4):449-55. doi:
 430 [http://dx.doi.org/10.1016/S1001-0742\(08\)62078-1](http://dx.doi.org/10.1016/S1001-0742(08)62078-1).

431 Liu, Lina, Hansong Chen, Peng Cai, Wei Liang, and Qiaoyun Huang. 2009. "Immobilization
 432 and phytotoxicity of Cd in contaminated soil amended with chicken manure compost."
 433 *Journal of Hazardous Materials* 163 (2–3):563-7. doi:
 434 <http://dx.doi.org/10.1016/j.jhazmat.2008.07.004>.

435 López-Millán, Ana-Flor, Ruth Sagardoy, María Solanas, Anunciación Abadía, and Javier
 436 Abadía. 2009. "Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown
 437 in hydroponics." *Environmental and Experimental Botany* 65 (2-3):376-85.

438 Martins, L. L., R. Reis, I. Moreira, F. Pinto, J. Sales, and M. P. Mourato. 2013.
 439 "Antioxidative response of plants to oxidative stress induced by Cadmium." In
 440 *Cadmium - Characteristics, Sources of Exposure, Health and Environmental Effects*,
 441 edited by Mirza Hasanuzzaman and Masayuki Fujita, 369. New York: Nova
 442 Publishers.

443 Mengel, Konrad. 2007. "Potassium." In *Handbook of Plant Nutrition*, edited by Allen V.
 444 Barker and David J. Pilbeam, 91-120. Boca Raton, FL, USA: CRC Press.

445 Michalska, Malgorzata, and Håkan Asp. 2001. "Influence of Lead and Cadmium on Growth,
 446 Heavy Metal Uptake and Nutrient Concentration of Three Lettuce Cultivars Grown in

- Hydroponic Culture." *Communications in Soil Science and Plant Analysis* 32 (3-4):571-83. doi: 10.1081/CSS-100103029.
- Mohamed, Ibrahim, Bocar Ahamadou, Ming Li, Changxiu Gong, Peng Cai, Wei Liang, and Qiaoyun Huang. 2010. "Fractionation of copper and cadmium and their binding with soil organic matter in a contaminated soil amended with organic materials." *Journal of Soils and Sediments* 10 (6):973-82. doi: 10.1007/s11368-010-0199-1.
- Morsch, V. M, and A. F. Martins. 1999. "Transfer of lead and cadmium from an organic fertilizer to vegetables." *Toxicological & Environmental Chemistry* 68 (1-2):105-15. doi: 10.1080/02772249909358649.
- Mourato, M., R. Reis, and L. Martins. 2012. "Characterization of Plant Antioxidative System in Response to Abiotic Stresses: A Focus on Heavy Metal Toxicity." In *Advances in Selected Plant Physiology Aspects*, edited by G. Montanaro and B. Dichio, 23-44. Rijeka: Intech.
- Mulligan, C. N., R. N. Yong, and B. F. Gibbs. 2001. "Remediation technologies for metal-contaminated soils and groundwater: an evaluation." *Engineering Geology* 60 (1-4):193-207. doi: [http://dx.doi.org/10.1016/S0013-7952\(00\)00101-0](http://dx.doi.org/10.1016/S0013-7952(00)00101-0).
- Nagajyoti, P., K. Lee, and T. Sreekanth. 2010. "Heavy metals, occurrence and toxicity for plants: a review." *Environmental Chemistry Letters* 8 (3):199-216. doi: 10.1007/s10311-010-0297-8.
- Narwal, R. P., and B. R. Singh. 1998. "Effect of Organic Materials on Partitioning, Extractability and Plant Uptake of Metals in an Alum Shale Soil." *Water, Air, and Soil Pollution* 103 (1-4):405-21. doi: 10.1023/A:1004912724284.
- Pinto, A. P., A. M. Mota, A. Varennes, and F. C. Pinto. 2004. "Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants." *Science of the Total Environment* 326:239-47.

- Pinto, Filipa R., Miguel P. Mourato, Joana R. Sales, Inês Neto Moreira, and Luisa Louro Martins. 2017. "Oxidative stress response in spinach plants induced by cadmium." *Journal of Plant Nutrition* 40 (2):268-76. doi: 10.1080/01904167.2016.1240186.
- Seth, C. S., T. Remans, E. Keunen, M. Jozefczak, H. Gielen, K. Opdenakker, N. Weyens, J. Vangronsveld, and A. Cuypers. 2012. "Phytoextraction of toxic metals: a central role for glutathione." *Plant Cell and Environment* 35 (2):334-46. doi: 10.1111/j.1365-3040.2011.02338.x.
- Sinha, Sarita, Shekhar Mallick, Rohit Kumar Misra, Sarita Singh, Ankita Basant, and Amit Kumar Gupta. 2007. "Uptake and translocation of metals in *Spinacia oleracea* L. grown on tannery sludge-amended and contaminated soils: Effect on lipid peroxidation, morpho-anatomical changes and antioxidants." *Chemosphere* 67 (1):176-87. doi: <http://dx.doi.org/10.1016/j.chemosphere.2006.08.026>.

491 **Table 1** Main characteristics of the soils at each experimental condition (value \pm standard deviation).
492 The asterisk indicates significant differences ($p < 0.05$) between the 0% CS and other CS levels, for
493 each Cd concentration.

Parameter	Cd Level	CS (g/100 g)			
		0	2.5	5	10
pH(H ₂ O)	No Cd	5.03 \pm 0.12	5.80 \pm 0.12*	6.75 \pm 0.13*	7.73 \pm 0.35*
	LC	6.48 \pm 0.10	6.49 \pm 0.11	6.98 \pm 0.10*	7.83 \pm 0.21*
	HC	6.58 \pm 0.10	6.40 \pm 0.08*	7.13 \pm 0.17*	7.90 \pm 0.20*
Conductivity (mS/cm)	No Cd	0.48 \pm 0.01	0.59 \pm 0.04	0.50 \pm 0.06	0.69 \pm 0.04*
	LC	0.43 \pm 0.07	0.51 \pm 0.09	0.47 \pm 0.08	0.75 \pm 0.04*
	HC	0.54 \pm 0.05	0.48 \pm 0.04	0.54 \pm 0.04	0.75 \pm 0.02*
Total carbon (%)	No Cd	0.16 \pm 0.01	0.20 \pm 0.02*	0.25 \pm 0.01*	0.38 \pm 0.02*
	LC	0.17 \pm 0.02	0.21 \pm 0.01*	0.26 \pm 0.01*	0.41 \pm 0.02*
	HC	0.17 \pm 0.01	0.22 \pm 0.02*	0.28 \pm 0.02*	0.41 \pm 0.02*
NH ₄ ⁺ (mg/kg)	No Cd	69.35 \pm 3.90	18.05 \pm 3.45*	2.30 \pm 0.55*	0.85 \pm 0.05*
	LC	87.90 \pm 10.20	27.60 \pm 3.05*	3.40 \pm 0.25*	1.60 \pm 0.30*
	HC	59.50 \pm 2.80	7.80 \pm 0.55*	2.10 \pm 0.30*	5.45 \pm 0.45*
NO ₃ ⁻ (mg/kg)	No Cd	486.6 \pm 63.4	466.9 \pm 75.9	243.1 \pm 12.6*	265.9 \pm 17.4*
	LC	562.5 \pm 41.4	398.7 \pm 32.6*	176.5 \pm 14.5*	223.9 \pm 19.5*
	HC	657.0 \pm 93.3	438.3 \pm 15.1*	257.4 \pm 2.9*	178.0 \pm 1.1*
P (mg/kg)	No Cd	52.5 \pm 2.3	129.4 \pm 13.7*	243.5 \pm 24.7*	446.6 \pm 53.6*
	LC	65.4 \pm 15.8	150.5 \pm 23.9*	241.2 \pm 26.7*	480.7 \pm 43.8*
	HC	51.6 \pm 12.4	139.5 \pm 16.8*	232.6 \pm 17.8*	410.2 \pm 32.8*
K (mg/kg)	No Cd	160.2 \pm 44.0	258.1 \pm 51.1*	383.1 \pm 46.8*	831.2 \pm 127.8*
	LC	160.2 \pm 16.6	323.6 \pm 38.7*	389.7 \pm 24.8*	408.8 \pm 39.0*
	HC	205.1 \pm 35.8	425.2 \pm 55.6*	513.8 \pm 83.6*	727.3 \pm 73.0*
Na (mg/kg)	No Cd	70.1 \pm 17.9	125.4 \pm 10.3*	129.3 \pm 26.2*	289.1 \pm 31.3*
	LC	82.3 \pm 22.9	153.7 \pm 24.1*	139.9 \pm 14.7*	264.0 \pm 37.8*
	HC	58.2 \pm 12.9	116.6 \pm 18.9*	168.4 \pm 16.9*	230.8 \pm 26.5*
Ca (mg/kg)	No Cd	99.8 \pm 16.8	143.4 \pm 33.0	158.8 \pm 24.7*	280.3 \pm 18.4*
	LC	109.5 \pm 20.3	143.1 \pm 21.7	175.8 \pm 9.8*	271.8 \pm 26.1*
	HC	121.0 \pm 10.5	153.5 \pm 25.7	189.9 \pm 13.6*	251.2 \pm 11.0*
Mg (mg/kg)	No Cd	39.7 \pm 6.8	50.1 \pm 9.2	59.3 \pm 8.2*	130.9 \pm 11.9*
	LC	26.9 \pm 6.7	49.6 \pm 6.4*	59.8 \pm 5.2*	123.8 \pm 12.6*
	HC	30.7 \pm 3.5	50.8 \pm 9.2*	70.7 \pm 9.5*	114.7 \pm 13.6*
Fe (mg/kg)	No Cd	6.6 \pm 1.0	9.3 \pm 0.8*	12.9 \pm 0.9*	21.3 \pm 1.3*
	LC	5.7 \pm 0.6	9.0 \pm 0.3*	12.4 \pm 0.8*	23.2 \pm 2.6*
	HC	4.7 \pm 0.3	10.3 \pm 0.9*	12.0 \pm 1.1*	23.7 \pm 2.8*
Cu (mg/kg)	No Cd	1.1 \pm 0.1	1.2 \pm 0.0	1.3 \pm 0.0*	1.5 \pm 0.1*
	LC	1.2 \pm 0.1	1.3 \pm 0.0	1.3 \pm 0.1	1.4 \pm 0.1*
	HC	1.2 \pm 0.0	1.3 \pm 0.1	1.3 \pm 0.1	1.4 \pm 0.1*
Zn (mg/kg)	No Cd	0.7 \pm 0.2	1.5 \pm 0.2*	2.2 \pm 0.4*	3.6 \pm 0.1*
	LC	0.8 \pm 0.0	2.0 \pm 0.4*	2.7 \pm 0.3*	4.3 \pm 0.5*
	HC	1.0 \pm 0.2	2.4 \pm 0.5*	3.0 \pm 0.6*	3.7 \pm 0.5*
Mn (mg/kg)	No Cd	1.5 \pm 0.2	3.6 \pm 0.8*	3.6 \pm 0.3*	6.3 \pm 0.7*
	LC	1.6 \pm 0.5	3.5 \pm 0.8*	3.8 \pm 0.8*	6.9 \pm 0.7*
	HC	1.8 \pm 0.5	3.0 \pm 0.5*	4.1 \pm 0.6*	5.9 \pm 0.6*

494

495

496

497 **Table 2** Main characteristics of the Cattle Slurry (CS) used in the experiment

Parameter	Value
Dry matter (g/kg)	11.74±0.55
Organic matter (g/kg)	7.20±0.48
pH	8.23±0.11
Conductivity (mS/cm)	17.25±0.70
N (g/kg)	3.45±0.86
P (g/kg)	0.85±0.03
Na (mg/kg)	1022.4±40.3
K (mg/kg)	3567.8±252.9
Ca (mg/kg)	2820.1±225.0
Mg (mg/kg)	665.3±15.6
Fe (mg/kg)	373.3±38.6
Cu (mg/kg)	2.87±0.14
Zn (mg/kg)	18.62±0.26
Mn (mg/kg)	27.41±1.96

498

499

500

501

502 **Table 3** Element concentration of spinach leaves (L), roots (R) and stems (S), only for Cd, at each experimental condition (value \pm standard
503 deviation), on a dry matter basis. Different lowercase letters represents significant differences ($p < 0.05$) between the Cd treatments at each CS
504 concentration, for each plant part

Element	Plant part	Treatment											
		CS0			CS2.5			CS5			CS10		
		No Cd	LC	HC	No Cd	LC	HC	No Cd	LC	HC	No Cd	LC	HC
Na (g/kg)	L	2.80 \pm 0.36 ^a	5.34 \pm 0.30 ^b	4.66 \pm 0.14 ^b	3.68 \pm 0.17 ^a	5.98 \pm 0.25 ^b	6.10 \pm 0.08 ^b	4.96 \pm 0.29 ^a	6.08 \pm 0.19 ^b	7.59 \pm 0.23 ^c	9.65 \pm 0.57 ^a	11.34 \pm 1.46 ^a	10.09 \pm 1.09 ^a
	R	0.80 \pm 0.09 ^a	1.45 \pm 0.39 ^a	1.45 \pm 0.39 ^a	3.39 \pm 0.31 ^a	3.42 \pm 0.20 ^a	2.41 \pm 0.22 ^b	4.98 \pm 0.24 ^a	4.01 \pm 0.20 ^a	4.65 \pm 0.67 ^a	9.93 \pm 1.55 ^a	8.63 \pm 0.71 ^a	8.15 \pm 0.37 ^a
K (g/kg)	L	52.0 \pm 2.3 ^a	35.4 \pm 2.0 ^b	35.8 \pm 2.0 ^b	72.3 \pm 8.0 ^a	65.6 \pm 0.9 ^a	63.4 \pm 3.5 ^a	80.6 \pm 5.8 ^{ab}	85.1 \pm 4.6 ^a	70.4 \pm 4.4 ^b	80.2 \pm 1.1 ^a	80.8 \pm 4.6 ^a	71.5 \pm 1.7 ^b
	R	7.88 \pm 1.87 ^a	8.42 \pm 2.51 ^a	6.48 \pm 3.0 ^a	39.3 \pm 2.7 ^a	29.6 \pm 3.6 ^b	17.0 \pm 4.3 ^c	47.9 \pm 2.5 ^a	28.6 \pm 3.0 ^b	25.3 \pm 4.9 ^b	45.8 \pm 3.7 ^a	41.8 \pm 5.1 ^a	29.5 \pm 1.0 ^b
Ca (g/kg)	L	2.77 \pm 0.10 ^a	2.36 \pm 0.06 ^b	2.73 \pm 0.20 ^a	1.85 \pm 0.20 ^a	1.88 \pm 0.07 ^a	2.68 \pm 0.04 ^b	1.22 \pm 0.09 ^a	1.35 \pm 0.15 ^a	1.92 \pm 0.21 ^b	0.96 \pm 0.09 ^a	1.07 \pm 0.10 ^a	1.04 \pm 0.10 ^a
	R	0.75 \pm 0.04 ^a	2.22 \pm 0.30 ^b	1.71 \pm 0.28 ^b	2.31 \pm 0.20 ^a	2.37 \pm 0.28 ^a	2.35 \pm 0.23 ^a	2.29 \pm 0.07 ^a	2.35 \pm 0.09 ^a	2.92 \pm 0.24 ^b	2.82 \pm 0.12 ^a	3.57 \pm 0.31 ^b	3.56 \pm 0.25 ^b
Mg (g/kg)	L	5.47 \pm 0.38 ^a	4.70 \pm 0.26 ^b	4.92 \pm 0.38 ^{ab}	8.47 \pm 0.51 ^a	7.85 \pm 0.20 ^a	6.80 \pm 0.22 ^b	7.24 \pm 0.24 ^{ab}	7.86 \pm 0.28 ^a	6.95 \pm 0.36 ^b	6.42 \pm 0.43 ^a	4.44 \pm 0.05 ^b	5.01 \pm 0.25 ^b
	R	1.02 \pm 0.11 ^a	2.35 \pm 0.39 ^b	1.31 \pm 0.28 ^a	6.79 \pm 0.14 ^a	4.56 \pm 0.39 ^b	3.60 \pm 0.07 ^c	7.45 \pm 0.25 ^a	5.47 \pm 0.03 ^b	6.04 \pm 0.12 ^c	6.85 \pm 0.29 ^a	5.68 \pm 0.07 ^b	5.65 \pm 0.17 ^b
Fe (mg/kg)	L	172 \pm 17 ^a	219 \pm 15 ^b	209 \pm 9 ^b	106 \pm 8 ^a	112 \pm 13 ^a	107 \pm 7 ^a	95 \pm 18 ^a	99 \pm 8 ^a	83 \pm 8 ^a	104 \pm 9 ^a	211 \pm 28 ^b	198 \pm 35 ^b
	R	1397 \pm 18 ^a	2391 \pm 283 ^b	2205 \pm 130 ^b	1169 \pm 115 ^a	1475 \pm 82 ^b	1502 \pm 62 ^b	1030 \pm 109 ^a	1303 \pm 53 ^b	1395 \pm 46 ^b	1241 \pm 121 ^a	1853 \pm 217 ^b	2789 \pm 435 ^c
Cu (mg/kg)	L	6.8 \pm 0.7 ^a	6.5 \pm 1.5 ^a	5.9 \pm 0.7 ^a	8.8 \pm 0.6 ^a	10.9 \pm 0.5 ^b	7.6 \pm 0.7 ^a	9.2 \pm 0.9 ^a	10.7 \pm 0.4 ^b	10.9 \pm 1.0 ^c	10.9 \pm 1.0 ^a	10.9 \pm 0.1 ^a	10.0 \pm 1.4 ^a
	R	7.7 \pm 1.7 ^a	28.1 \pm 3.4 ^b	19.6 \pm 2.2 ^c	23.3 \pm 1.6 ^a	29.3 \pm 2.1 ^b	26.2 \pm 0.7 ^{ab}	23.1 \pm 0.8 ^a	25.5 \pm 0.5 ^b	31.9 \pm 0.9 ^c	42.8 \pm 3.0 ^a	43.3 \pm 5.4 ^a	56.8 \pm 5.8 ^b
Mn (mg/kg)	L	127.6 \pm 13.0 ^a	92.01 \pm 2.5 ^b	259.1 \pm 5.9 ^c	56.9 \pm 8.7 ^a	116.6 \pm 5.6 ^b	201.0 \pm 6.7 ^c	53.4 \pm 10.1 ^a	88.2 \pm 1.5 ^b	98.9 \pm 10.4 ^b	113.3 \pm 11.9 ^a	107.5 \pm 11.4 ^a	104.2 \pm 14.7 ^a
	R	44.0 \pm 3.0 ^a	32.1 \pm 10.7 ^a	103.1 \pm 15.4 ^b	129.1 \pm 12.3 ^a	255.8 \pm 10.6 ^b	236.1 \pm 9.6 ^b	222.2 \pm 26.6 ^{ab}	257.4 \pm 5.9 ^a	187.5 \pm 7.1 ^b	400.4 \pm 44.0 ^a	391.2 \pm 67.5 ^a	467.3 \pm 81.5 ^a
Zn (mg/kg)	L	133.4 \pm 8.9 ^a	56.4 \pm 4.7 ^b	53.0 \pm 1.2 ^b	97.4 \pm 8.7 ^a	74.7 \pm 4.0 ^b	44.6 \pm 0.3 ^c	118.4 \pm 8.1 ^a	102.7 \pm 8.3 ^a	40.9 \pm 1.2 ^b	60.0 \pm 3.6 ^a	87.0 \pm 5.2 ^b	65.3 \pm 5.6 ^a
	R	53.5 \pm 5.1 ^a	6.8 \pm 32.7	15.0 \pm 3.1 ^b	114.7 \pm 10.4 ^a	61.7 \pm 9.3 ^b	44.8 \pm 5.6 ^b	133.9 \pm 8.8 ^a	97.8 \pm 11.7 ^b	45.7 \pm 4.3 ^c	68.2 \pm 5.7 ^a	62.1 \pm 4.5 ^{ab}	51.7 \pm 3.4 ^b
Cd (mg/kg)	L	0.8 \pm 0.1 ^a	87.3 \pm 12.1 ^b	317.4 \pm 2.6 ^c	0.6 \pm 0.1 ^a	184.4 \pm 4.1 ^b	367.7 \pm 6.4 ^c	0.6 \pm 0.2 ^a	140.8 \pm 4.8 ^b	304.5 \pm 35.4 ^c	0.4 \pm 0.1 ^a	26.6 \pm 1.0 ^b	63.1 \pm 2.2 ^c
	S	0.7 \pm 0.1 ^a	89.7 \pm 9.4 ^b	240.2 \pm 9.9 ^c	0.5 \pm 0.1 ^a	150.7 \pm 1.9 ^b	211.7 \pm 14.1 ^c	0.7 \pm 0.0 ^a	127.8 \pm 3.1 ^b	173.0 \pm 12.1 ^c	0.5 \pm 0.0 ^a	41.7 \pm 5.1 ^b	67.6 \pm 2.6 ^c
	R	1.2 \pm 0.2 ^a	152.6 \pm 10.7 ^b	340.8 \pm 49.7 ^c	2.8 \pm 0.7 ^z	330.6 \pm 10.1 ^b	502.4 \pm 15.5 ^c	2.5 \pm 0.4 ^a	285.5 \pm 10.1 ^b	520.5 \pm 20.1 ^c	0.9 \pm 0.1 ^a	82.2 \pm 1.8 ^b	222.9 \pm 15.7 ^c

Figures

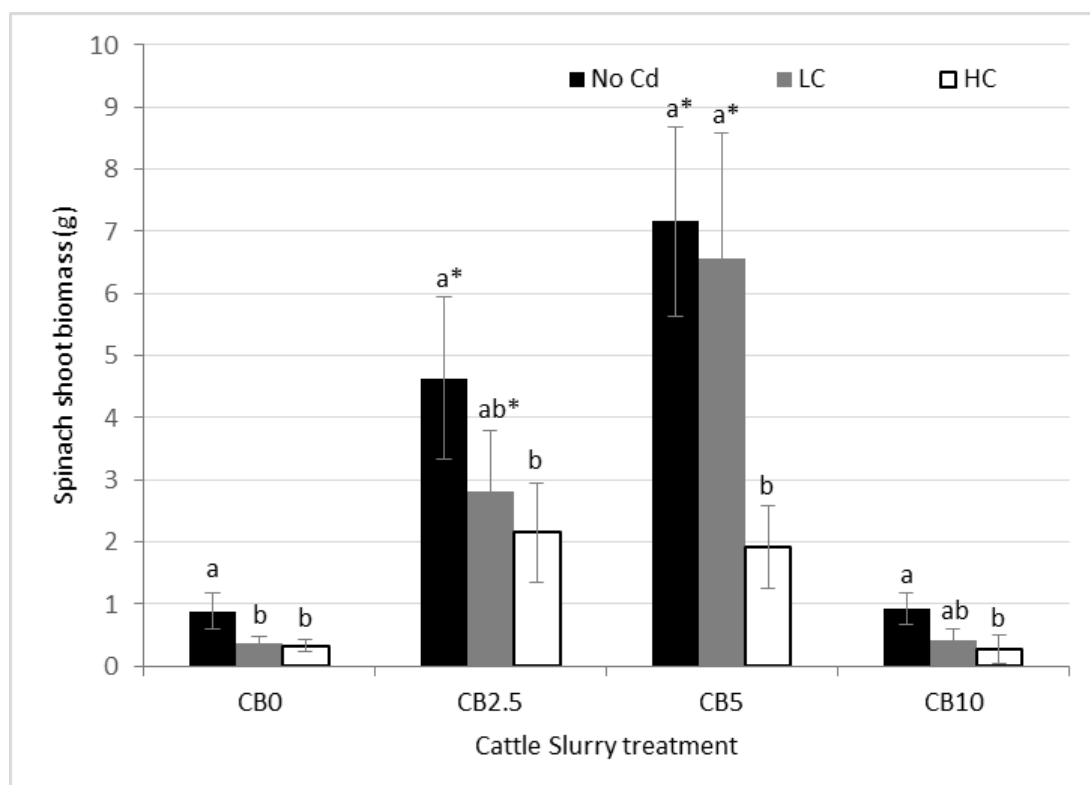
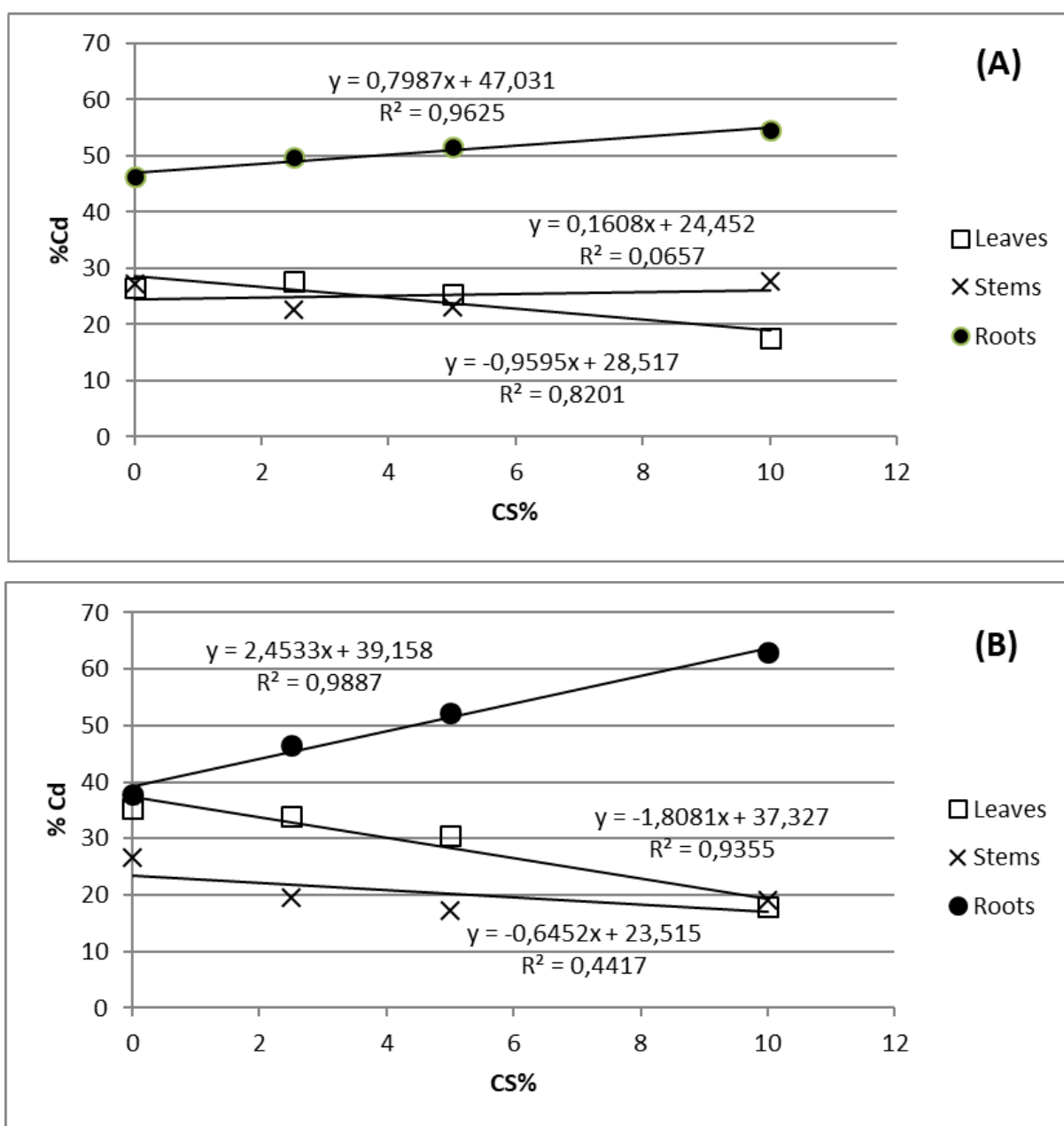


Fig. 1 Spinach shoot biomass obtained seventy days after germination. Error bars represent \pm standard deviation and the asterisk indicates significant differences ($p < 0.05$) between the different CS treatments in relation to control. Different lowercase letters represents significant differences ($p < 0.05$) between the Cd treatments at each CS concentration

519



520

521

522 **Fig. 2** Percentage of Cd in each plant part (leaves, stems and roots), in relation to the total, as
 523 a function of CS treatment for both Cd treatments, A - LC and B - HC

524

525

526

527